AGENDA
Diaprepes Task Force/Citrus Growers Meeting
May 12, 2005

University of Florida, IFAS
Southwest Florida REC
2686 Highway 29 N
Immokalee, FL 34142-9515

Conveners: Larry Duncan and Phil Stansly

9:00 a.m.	**Welcome and Introductions**	Garvie Hall, *Chair* Constance Riherd, *Vice Chair*
	Symposium: Novel IPM Tactics	
9:15	Insect enzyme systems and how to exploit them.	Charles A. Powell
9:40	The how's and why's of developing transgenic citrus with Diaprepes root weevil resistance.	Robert G. Shatters, Jr.
10:05	**Break**	
	Research Updates	
10:20	A visual presentation of the spatiotemporal aspects of Diaprepes abundance in a small east coast citrus grove.	R.C. Adair, Jr. and G.K. Ross
10:35	Progress in the development of new rootstocks tolerant of the *Diaprepes/Phytophthora* Complex.	J.W. Grosser, J.H. Graham, C.W. McCoy, A. Hoyte, H.M. Rubio, and J.L. Chandler
10:50	*Diaprepes, Phytophthora*, and hurricanes: Differential growth and survival of Hamlin orange trees budded to five rootstocks in an experimental grove.	R.J. Stuart, C.W. McCoy, and W.S. Castle
11:05	Management of the Phytophthora-Diaprepes (PD) Complex is site specific.	J.H. Graham, L.W. Duncan, C.W. McCoy, and R.C. Adair, Jr.
11:20	Diaprepes root weevil on ornamentals - An update from Southern Florida.	C.M. Mannion, A. Diaz, and H. Glenn
11:35	An update on Diaprepes egg parasitoid research.	B.J. Ulmer, J.E. Peña, C.W. McCoy, R.E. Duncan, S.L. Lapointe, and D.G. Hall
11:50	Feeding and oviposition behavior of *Diaprepes abbreviatus* (L.) (Coleoptera: Curculionidae) on peach and sweet orange in Puerto Rico.	A. Delgado-Sánchez, A.L. González-Rodríguez, P. Stansly, M. Del Carmen Libran, and R.E. Rouse

12:05 **General Discussion**

12:30 **Lunch**

1:30-3:30 **Poster Session**

Some chemical and morphological comparisons of laboratory-reared and field-collected adult Diaprepes root weevil.	D.G. Hall and R. Alessandro. USDA, ARS, U.S. Horticultural Research Lab
Susceptibility of *Diaprepes abbreviatus* (Coleoptera: Curculionidae) to novel isolates of *Bacillus thuringiensis*.	A.A. Weathersbee III and S.L. Lapointe. USDA, ARS, U.S. Horticultural Research Lab
Reproductive biology of *Fidiobia dominica* (Hymenoptera: Platygastridae), a new exotic parasitoid of *Diaprepes abbreviatus* (Coleoptera: Curculionidae).	R.E. Duncan and J.E. Peña. UF-IFAS Tropical REC
Successful oviposition and reproductive biology of *Aprostocetus vaquitarum* (Hymenoptera: Eulophidae): A predator of *Diaprepes abbreviatus* (Coleoptera: Curculionidae).	J.A. Jacas[1], J.E. Peña[2] and R.E. Duncan.[2] [1]Universitat Jaume I, Dept. Ciencies Experimentals, Castello de la Plana, Spain; [2]UF-IFAS Tropical REC
Toxicity of pesticides used in citrus to *Aprostocetus vaquitarum* (Hymenoptera: Eulophidae), an egg parasitoid of *Diaprepes abbreviatus* (Coleoptera: Curculionidae).	B.J. Ulmer,[1] J.E. Peña,[1] R.E. Duncan,[1] and S.L. Lapointe.[2] [1]UF-IFAS Tropical REC; [2]USDA, ARS, U.S. Horticultural Research Lab
Delta-9 Desaturase from the Diaprepes root weevil.	C.S. Katsar, W.B. Hunter, and S.L. Lapointe. USDA, ARS, U.S. Horticultural Research Lab
Results of a three-year-field study of Surround® particle film for control of the Diaprepes root weevil.	S.L. Lapointe and C.L. McKenzie. USDA, ARS, U.S. Horticultural Research Lab
IPM using entomopathogenic nematodes: The roles of longevity and adaptation.	L.W. Duncan,[1] K. Nguyen,[2] and F.E. El-Borai.[1] [1]UF-IFAS, Citrus REC; [2]Department of Entomology and Nematology, Gainesville
Toward circumventing non-target effects of augmenting entomopathogenic nematode communities.	L.W. Duncan,[1] J.H. Graham,[1] F. El-Borai,[1] and D.L. Porazinska.[2] [1]UF-IFAS, Citrus REC; Lauderdale REC, Fort Lauderdale, FL
Ant Predation on Neonate Larvae of the Root Weevil, *Diaprepes abbreviatus*: A Manipulative Field Experiment.	R.J. Stuart and C.W. McCoy. UF-IFAS, Citrus REC

Insect Enzyme Systems and How to Exploit Them. *C. Powell,[1] R. Shatters,[2] and D. Borovsky[3]. [1]UF-IFAS, Indian River REC, Fort Pierce, FL 34945. [2]USDA ARS, U.S. Horticulture Research Lab, Fort Pierce, FL 34945. [3]UF, FMEL, Vero Beach, FL 32962.*

Organisms require enzymes to digest their food. In insects there are two basic digestive enzyme systems: serine proteases, including trypsin and chymotrypsin, and cysteine proteases, primarily cathepsins. These enzymes are under regulatory control so they are only active when there is substrate (food) present. In mosquitos, which use primarily serine proteases, regulation occurs via a hormone (trypsin modulating oostatic factor, TMOF). When the gut is empty, TMOF is secreted by the ovaries (adults) or brain (larvae) and there is little trypsin activity. When a meal is taken, TMOF decreases, trypsin activity increases, and the proteins in the meal are digested. If TMOF is present in the food (algae or yeast transformed to express TMOF), the larval trypsin activity is greatly reduced, they cannot digest the food, and they die. This product can now be used as part of an integrated strategy to control mosquitos.

We have learned that a TMOF-like regulatory process is conserved among a large variety of insects including those that feed on plants. Both tobacco bud worm (*Heliothis virescens*) and citrus root weevil (*Diaprepes abbreviatus*) larvae respond to the presence of TMOF in their artificial diets or on leaves with a better than 50% decrease in trypsin activity. This results in a 20 to 30% reduction in larval growth for both these insects, which would make them non-competitive in nature. Alfalfa has been transformed to express TMOF as a possible method to deliver the hormone to plant feeding insects. Heliothis larvae that feed on the transformed alfalfa show a reduction in leaf damage of 22 to 64% depending on the alfalfa line. Although the transgenic alfalfa reduced Diaprepes larval trypsin activity by up to 45%, the effect on larval growth was variable and not as great as the effect on Heliothis larvae. This is likely because Diaprepes has both serine and cysteine protease systems. We have discovered that the cysteine proteases are regulated by a chaperone protein that presumably allows the cathepsin to fold properly in the gut environment. RNAi technology (using double-stranded RNA to specifically silence a gene) has been used to inhibit the cysteine proteases. An RNAi complementary to a portion of the chaperone protein gene reduced Diaprepes cathepsin L activity to near zero, and resulted in a 33% reduction in larval weight gain. Thus, the technology has been developed to inhibit both Diaprepes digestive enzyme systems.

The How's and Why's of Developing Transgenic Citrus with Diaprepes Root Weevil Resistance. *R.G. Shatters, Jr. USDA, ARS, U.S. Horticultural Research Lab, Fort Pierce, FL 34945.*

Development of Diaprepes root weevil resistant citrus offers great benefit to the Florida citrus growers because it would eliminate the losses caused by this pest without the added cost of expensive management practices. However, despite extensive screening of existing citrus varieties, no natural resistance to the DRW exists in citrus. Biotechnology offers the advantage of introducing novel pieces of genetic material (genes) borrowed from other organisms that, when inserted into the citrus genome, encode for molecules that reduce or eliminate Diaprepes root weevil feeding damage. This could be accomplished using genes that encode toxins that directly kill the insect, inhibitory molecules that block the ability of the insect to extract nutrition from citrus roots, or deterrent molecules that prevent the insect from feeding on the roots. This is a proven strategy in the production of a growing number of commercial crops, and success stories using this strategy will be described. Specific hurdles that have to be overcome to apply this technology to citrus will also be presented.

A Visual Presentation of the Spatiotemporal Aspects of Diaprepes Abundance in a Small East Coast Citrus Grove.* *G.K. Ross and R.C. Adair. Florida Research Center for Agricultural Sustainability, Vero Beach, FL 32966.*

Little is known about the spatial distribution patterns or the rate and direction of the spread of Diaprepes infestations in citrus. Weekly adult weevil abundance data obtained from geo-referenced Tedders Traps placed in a diamond-shaped grid were projected geographically by means of a Geographic Information System (GIS). The GIS (ESRI ArcGIS) was used to provide spatial interpolation of Diaprepes abundance weekly for a period of one to four years (2000-2003) and video software (Macromedia Flash) combined the weekly interpolations into a video that enabled visualization of this spatiotemporal data. In addition, this data was synchronously aligned with weekly rainfall. The resultant video animation successfully portrayed different geographic and seasonal aspects of Diaprepes activity while simultaneously observing rainfall in a smooth transition through space and time than was previously thought possible. The visualization of the spatiotemporal aspects of adult Diaprepes captured by the Tedders traps was much more evident when viewed as an animated video than could be derived by more conventional analysis of tabular or graph data. The ability to view additional factors such as rainfall or soil temperature in conjunction with visualization of animated spatiotemporal data could not only elucidate the ecology of Diaprepes, but also provide essential timing and exact location information necessary for precision application of crop protectants for a fully Integrated Pest Management program for Diaprepes.

**This research was funded by the Florida Research Center for Agricultural Sustainability, Inc., a Tax Exempt 501(c)(3) non-profit research foundation.*

Progress in the Development of New Rootstocks Tolerant of the *Diaprepes/Phytophthora* Complex. *J.W. Grosser, J.H. Graham, C.W. McCoy, A. Hoyte, H.M. Rubio, and J.L. Chandler. UF-IFAS, Citrus REC, Lake Alfred, FL 33850.*

Our primary strategy for dealing with the Diaprepes/Phytophthora problem has been to develop complex rootstock hybrids that have the capacity to tolerate mechanical damage caused by weevil feeding and then recovery by exhibiting vigorous root growth in challenging soils inoculated with both *Phytophthora nicotianae* and *P. palmivora*. Annual crosses are being made of superior allotetraploid somatic hybrid rootstocks and resulting seed are planted in a high pH calcareous 'Winder' soil inoculated with both *Phytophthora spp*. in greenhouse flats. Vigorous healthy "tetrazyg" seedlings are selected and propagated by grafting to vigorous rootstocks and subsequently rooted cuttings. Crosses of pummelo x mandarin at the diploid level have also been included in the program the past two years. Selected new allotetraploid mandarin + pummelo somatic hybrids (potential sour orange replacements) are also being included in the assays. Replicated *Diaprepes* force-feeding assays are conducted in conetainers®, and hybrids selected for reduced mechanical damage are replanted in a 'Winder'/*Phytophthora* mix to assess recovery potential. Several hybrids have shown excellent capacity for complete recovery in this greenhouse test and are now being propagated for more extensive field evaluation; and one hybrid of Nova+HBpummelo x Cleo+Argentine trifoliate orange (orange #19) showed superior performance in two consecutive tests. Cuttings of this hybrid root easily, and several hundred have been produced for further evaluation. The first field test of greenhouse selected tetrazygs will be planted this spring in the Kelly block (via R. Stuart/C.W. McCoy), in efforts to validate the greenhouse screening program. Hamlin on replicates of 14 new rootstock candidates with a range of performance in the greenhouse tests will be included. 2004 greenhouse screens identified two promising hybrids of Nova+HBpummelo from open pollination, and several superior seedlings from Chandler pummelo that are being propagated for further evaluation. Cuttings from over 50 preselected hybrids (mostly from 2003 crosses) were produced during 2004, and the 2005 greenhouse screen is underway. Numerous preselected hybrids from 2004 crosses will be propagated by cuttings this summer for the 2006 screen. Citrus rootstock breeding and selection at the tetraploid level maximizes genetic diversity and selection efficiency, and shows great promise for generating new rootstocks that can tolerate the *Diaprepes/Phytophthora* complex.

***Diaprepes, Phytophthora*, and Hurricanes: Differential Growth and Survival of Hamlin Orange Trees Budded to Five Rootstocks in an Experimental Grove.** *R.J. Stuart, C.W. McCoy, and W.S. Castle. UF-IFAS, Citrus REC, Lake Alfred, FL 33850.*

The Diaprepes root weevil (DRW), *Diaprepes abbreviatus* (L.), in combination with *Phytophthora* spp. causes one of the most severe decline syndromes known in Florida citrus. We are comparing the growth and survival of Hamlin orange trees budded to five rootstocks (C-22, C-32, and C-35 citrange, Cleopatra mandarin, Swingle citrumelo) in a grove located on a poorly drained clay-loam soil near Poinciana, Osceola County, FL. Both DRW and *P. nicotianae* are present and catastrophic tree decline is symptomatic. The trees were planted in September 2001, and half of the trees receive foliar and soil-applied pesticides for DRW suppression whereas the remaining trees receive no chemical treatment except for routine irrigation, weed control, and fertilizer applications. In evaluations conducted during the summers of 2003 and 2004, trees receiving chemical protection had a faster growth rate (except Cleopatra mandarin), larger tree canopies, fewer adult DRW, and less tree decline than untreated trees. Trees on C-32, C-35, and Swingle citrumelo also tended to have higher growth rates, canopy volumes, and DRW populations but less tree decline than those on C-22 and Cleopatra mandarin. In this grove, root injury by DRW larvae appears to facilitate infection by *P. nicotianae*, and the combination of DRW and *P. nicotianae* is the primary cause of tree decline. In the summer of 2004, three hurricanes passed through central Florida and caused considerable wind and flooding damage to the experimental grove. Overall, 38.5% of the young trees sustained significant wind damage (i.e., uprooted, split trunks, broken branches). Trees receiving chemical treatment for DRW control suffered a disproportionate amount of wind damage (52.4%) compared to untreated trees (24.7%), probably because they had larger canopies and healthier root systems. The rootstock experiment at this site is continuing, and further studies are planned to evaluate newly developed rootstocks that might be especially suited to the pest and pathogen problems represented at this grove.

Management of the Phytophthora-Diaprepes (PD) Complex is Site Specific.* *J.H. Graham,[1] L.W. Duncan,[1] C.W. McCoy,[1] and R.C. Adair.[2] [1]UF-IFAS, Citrus REC, Lake Alfred, FL 33850. [2]Florida Research Center for Agricultural Sustainability, Vero Beach, FL 32966.*

The complex of *Phytophthora* spp. with structural root damage caused by larvae of Diaprepes root weevil (DRW) is primarily managed with rootstock resistance to the fungus because substantial resistance to larval feeding is not available. IPM of DRW can be intensive and expensive, so proper rootstock management is crucial to the success of weevil control measures. Most prominent site-specific characteristics that affect IPM are: 1) the grove location in central ridge vs. flatwoods, 2) the presence of optimum or marginal soils for the rootstocks, and 3) the prevalence of *P. palmivora*. In a central ridge grove on deep well-drained, entisol soil with a long history of DRW infestation, trees on Carrizo citrange maintain tolerance to *P. nicotianae* as long as weevil activity and rainfall are not excessive. In a central Florida flatwoods site, with poorly drained, acid alfisol of sand muck over clay and very high DRW prevalence, 3.5-yr-old trees on rootstocks Swingle and Citrange C-32, C-35 sustain resistance to *P. nicotianae* despite a periodically high water table. Tree decline caused by PD complex is lower on resistant rootstocks than susceptible rootstocks, Cleopatra mandarin, and Citrange C-22. Insecticides reduce young tree decline even further, confirming the primacy of DRW control for tolerance to PD complex. On the east coast, fine textured alfisols, Winder and Manatee, promote damaging levels of DRW and *P. palmivora* as well as *P. nicotianae*. Despite a Ridomil fungicide program (2 applications per season), 10-yr-old trees on Swingle show substantial tree decline from 1999-2004, while health of trees on Cleopatra is maintained at a much higher level than Swingle. Swingle is uniquely susceptible to *P. palmivora* under certain site conditions. Resistance to *Phytophthora* spp. and tolerance to the PD complex are the foundation for site-specific tactics to control DRW and *Phytophthora* spp.

[*] *This research was supported by grants from the FCPRAC, USDA-CSREES, and Syngenta Crop Protection.*

Diaprepes Root Weevil on Ornamentals – An Update from Southern Florida. *C. Mannion, A. Diaz, and H. Glenn. UF-IFAS, Tropical REC, Homestead, FL 33031.*

The Diaprepes root weevil, *Diaprepes abbreviatus* (L.) (Coleoptera: Curculionidae) is a serious pest problem facing citrus growers in Florida and has also become a severe problem for ornamental plant growers. Most research with this weevil has focused on citrus, and there has been relatively little research on Diaprepes root weevil as a pest of ornamental crops. There are numerous concerns about this pest, which include detection and monitoring of soil-inhabiting stages, the destructive habits of larvae and adults, the risk of spreading the pest with infested plant material, and the impact of damage caused by this pest on the ornamental plants. Studies were conducted to examine the effects of root feeding of Diaprepes root weevil and flooding on three commonly grown ornamental plants grown in southern Florida [buttonwood (*Conocarpus erectus*), oak (*Quercus virginiana*), and pigmy palm (*Phoenix roebelenii*)]. These plant species are known to support larval and adult stages of Diaprepes root weevil and buttonwood has been shown to be a preferred host. Host plants in containers were exposed to larvae and/or flooding conditions to evaluate the impact on plant growth and condition and net photosynthesis (determined on a leaf area), transpiration, and water-use efficiency. Leaf photosynthesis was reduced at temperatures less than approximately 21°C regardless of treatment. Tolerance to root feeding, flooding, and the combination of both differed among host plants. In some host plants, photosynthesis, root growth, tree diameter, and height were reduced due to root damage caused by larval feeding. Flooding negatively impacted plant growth and photosynthesis; however, the combination of root feeding and flooding did not appear to have an additive or synergistic affect on the host plants tested. Studies are ongoing to monitor Diaprepes root weevil populations in a commercial field nursery comprised primarily of native plants. Adult weevil populations are monitored using modified Teddars traps and making visual counts on plant foliage on a weekly basis. Relatively high numbers of adult weevils are trapped; however, more weevils were observed on the trees than collected in the traps. Peak populations were seen in the late fall (December) and late spring (April-May). During peak populations, the type of tree closest to the trap may influence the number of weevils caught. Studies were conducted to evaluate establishment of neonates in container ornamental plants by attaching varying numbers of egg masses to foliage of container plants and determining the number of neonates that became established in these containers. Establishment of neonates was very poor. Less than 1% of neonates were able to establish in container plants regardless of the number of egg masses on the plant. No neonate larvae survived in containers that had previously been drenched with bifenthrin (Talstar) at the rate of 25 ppm. Although neonates have been shown to be very active and capable of moving out of container plants, no larvae were found in control plants that where in close proximity to plants with egg masses.

An Update on Diaprepes Egg Parasitoid Research. *B.J. Ulmer,[1] J.E. Peña,[1] C.W. McCoy,[2] R.E. Duncan,[1] S.L. Lapointe,[3] and D.G. Hall[3]. [1]UF-IFAS Tropical REC, Homestead, FL 33031. [2]UF-IFAS Citrus REC, Lake Alfred, FL 33850. [3]USDA, ARS, U.S. Horticulture Research Lab, Fort Pierce, FL 34945.*

Quadrastichus haitiensis and *Aprostocetus vaquitarum* are established in Miami-Dade county where they have been consistently recovered resulting in the mortality of approximately 60% of *Diaprepes abbreviatus* eggs. *A. vaquitarum* is also established in Broward county and is being recovered in Glades county. Both parasitoids have been sporadically recovered in St. Lucie county. The influence of temperature on the life history traits of *Q. haitiensis* and *A. vaquitarum* was investigated in the laboratory. Development from egg to adult was fastest for both species at 25-30°C; no development was observed for either species from 5-15°C. Oviposition was also highest at 25 and 30°C, reduced at both higher and lower temperatures for both species. Both species suffered moderate levels of mortality during brief exposure to 0°C at various periods of development but never suffered 100% mortality. Both *Q. haitiensis* and *A. vaquitarum* were most prolific when parasitizing 1- to 4-d-old host eggs. Very few adult parasitoids emerged from host eggs that were 5 to 7 d old. However, when exposed to *Q. haitiensis*, *D. abbreviatus* egg mortality was similar at all ages. Twelve pesticides used in citrus were tested for their toxicity to *A. vaquitarum*. Sevin 80WSP, Malathion 5EC, and Imidan 70WSB were the most detrimental; Admire 2F, Danitol 2.4EC, and Surround WP were also very harmful. Kocide 101WP, Citrus Soluble Oil, Micromite 80WGS, Acramite 50WS, Micromite 80WGS + Oil, Aliette WDG and Agrimek 0.15EC + Oil were less toxic to harmless. Studies have also been initiated in quarantine with *Fidiobia dominica* and *Haeckeliania* n. sp., collected in Dominica in 2003. Petitions for release from quarantine have been sent to APHIS PPQ. They are being reviewed by Mexico and Canada; we are waiting to hear from them.

Feeding and Oviposition Behavior of *Diaprepes abbreviatus* (L.) (Coleoptera: Curculionidae) on Peach and Sweet Orange in Puerto Rico. *A. Delgado-Sánchez[1,2] A.L. González-Rodríguez,[1] P. Stansly,[2] M. Del Carmen Libran,[1] and R.E. Rouse.[2] [1]Department of Crop Protection, University of Puerto Rico-Mayaguez. [2]UF-IFAS, Southwest Florida REC, Immokalee, FL 34142.*

Peach cultivars are being evaluated for their adaptation to the conditions of the central region of Puerto Rico where the root weevil, *Diaprepes abbreviatus* (L.), is endemic. *D. abbreviatus* has been reported as a pest of peaches in Florida and so would naturally be expected to attack peaches in Puerto Rico. However, preliminary field observations showed only minimal damage to new peach plantings in two locations where *D. abbreviatus* is abundant. Therefore, we embarked on a study to evaluate the preference and performance of *D. abbreviatus* on peach compared to sweet orange. Adults fed significantly more on Navel orange leaf disks than on peach disks in both choice and no-choice tests. Oviposition occurred on both peach cultivars tested, but more egg masses were laid on Navel orange leaf strips in the no-choice test. However, given the choice, adults preferred to oviposit on peach leaf strips while fed on Navel orange leaf strips although this behavior was reversed in some replications. Larval feeding damage 90 days after infestation was severe on roots of Cleopatra mandarin in 18.7 liter containers. Most of the cortex tissue on the primary root was removed and growth of roots and foliage was reduced. Larval feeding was also observed on peach roots, but there was no sign of growth reduction on foliage or roots compared to the control. These preliminary results indicate that *D. abbreviatus* will probably not be a primary pest of peach in Puerto Rico.

Some Chemical and Morphological Comparisons of Laboratory-Reared and Field-Collected Adult Diaprepes Root Weevil. *D.G. Hall and R. Alessandro. USDA, ARS, U.S. Horticultural Research Lab, Fort Pierce, FL 34945.*

Chemical and morphological comparisons showed differences may exist between laboratory-reared and field-collected adult Diaprepes root weevils and between field-collected weevils from different host plants in different geographical areas in Florida. Lab-reared weevils were larger in body size and displayed different chromatographic profiles and spectra of metabolites and lipids. Weevils field-collected from an ornamental nursery in Homestead were generally smaller than weevils field-collected from citrus in the Fort Pierce area. Chemical and morphological differences were attributed to differences during larval development in food-source nutrients, environmental conditions, and varying soil/media factors. The significance of these differences remain to be determined. Researchers using laboratory-reared weevils to make inferences for populations of wild weevils should keep such possible differences in mind.

Susceptibility of *Diaprepes abbreviatus* (Coleoptera: Curculionidae) to novel isolates of *Bacillus thuringiensis*. *A.A. Weathersbee and S.L. Lapointe. USDA, ARS, U.S. Horticulture Research Lab, Fort Pierce, FL 34945.*

Bacterial entomopathogens have not been adequately investigated as potential microbial agents for control of the citrus root weevil, *Diaprepes abbreviatus*. The bacterium, *Bacillus thuringiensis*, is known to be pathogenic to many insect pests but relatively few strains are active against Coleoptera. Endotoxins produced by the bacterium are activated in the gut of susceptible hosts upon ingestion, and can cause feeding inhibition, infection of host tissues, and eventual death. Researchers at the U.S. Horticultural Research Laboratory have assembled two collections of *B. thuringiensis* isolates that are plausibly active against *D. abbreviatus*. One collection contains patented isolates that demonstrate activity against Coleoptera. The other collection is comprised of isolates obtained from diseased larvae of *D. abbreviatus* collected in Florida citrus groves. Isolates from both collections are being screened for activity against neonatal and 3- to 4-week-old larvae of *D. abbreviatus*. Effective rates are determined for isolates that demonstrate activity in initial screening experiments. Application strategies developed for particularly promising isolates may include transgenic approaches as deemed appropriate.

Reproductive Biology of *Fidiobia dominica* (Hymenoptera: Platygastridae), a New Exotic Parasitoid of *Diaprepes abbreviatus* (Coleoptera: Curculionidae). *R.E. Duncan and J.E. Peña. UF-IFAS, Tropical REC, Homestead FL 33031.*

Fidiobia dominica Evans and Peña is a new species reared from *Diaprepes* spp. eggs collected in Dominica. It was imported into the quarantine facility in Homestead, Florida as a biocontrol agent for *Diaprepes abbreviatus*. The reproductive biology of *F. dominica* was studied in the laboratory using *D. abbreviatus* as a host. The developmental time for *F. dominica* from egg to adult at 25^{o}C was 20.3 ± 0.6 d (range, 18-25). Mean longevity of adults exposed to hosts was 8.1 d (range 6-13 d). The mean total progeny produced was 25.3, 8.2 male and 17.1 female offspring per female. Eighty five percent of fecundity occurred in the first 2 d of a females' lifetime. The total mortality caused by *F. dominica* to *D. abbreviatus* eggs was 39.5 per female.

Successful Oviposition and Reproductive Biology of *Aprostocetus vaquitarum* (Hymenoptera: Eulophidae): A Predator of *Diaprepes abbreviatus* (Coleoptera: Curculionidae). *J.A. Jacas,[1] J.E. Peña,[2] and R.E. Duncan.[2] [1]Universitat Jaume I, Departament de Ciències Experimentals, Campus del Riu Sec, E-12071-Castelló de la Plana, Spain. [2]UF-IFAS, Tropical REC, Homestead, FL 33031.*

Aprostocetus vaquitarum (Wolcott) causes 78-91% mortality to eggs of *Diaprepes abbreviatus* (L.) under field conditions in southern Florida. In the laboratory, *A. vaquitarum* was reared on *D. abbreviatus* eggs at 25°C, a photoperiod of 12:12 (L:D) and with abundant hosts, *A. vaquitarum* adult females lived around 15 d. Oviposition on egg masses aged 0-3 d was significantly higher than on those aged 4-6 d. The mean number of eggs deposited per female was around 53, with extreme values of 124 and 19 eggs per female. Using these data in combination with the sex ratio observed in the field (0.16) and the duration of the preimaginal stages, r_m (0.168-0.142 d^{-1}), T (22.39-22.89 d) and R_o (43.03-25.81 females per female) were calculated.

Toxicity of Pesticides Used in Citrus to *Aprostocetus vaquitarum* (Hymenoptera: Eulophidae), an Egg Parasitoid of *Diaprepes abbreviatus* (Coleoptera: Curculionidae). *B.J. Ulmer,[1] J.E. Peña,[1] R.E. Duncan,[1] S.L. Lapointe.[2] [1]UF-IFAS Tropical REC, Homestead, FL 33031. [2]USDA, ARS, U.S. Horticulture Research Lab, Fort Pierce, FL 34945.*

Twelve pesticides used in citrus were tested in the laboratory for their toxicity to *Aprostocetus vaquitarum,* an egg parasitoid of *Diaprepes abbreviatus*. Sevin 80WSP, Malathion 5EC, and Imidan 70WSB resulted in the most rapid death of *A. vaquitarum*. Admire 2F, Danitol 2.4EC, and Surround WP were also very harmful. Kocide 101WP, Citrus Soluble Oil, Micromite 80WGS, Acramite 50WS, Micromite 80WGS + Oil, Aliette WDG and Agrimek 0.15EC + Oil were not significantly different from the control. Sevin 80WSP, Malathion 5EC, and Imidan 70WSB also caused the highest mortality after 96 hours, followed by Admire 2F and Danitol 2.4EC. The relative toxicity of the pesticides was consistent over a 4-week period when aged out of doors protected from precipitation and sunlight. Significantly fewer adult *A. vaquitarum* emerged from *D. abbreviatus* eggs laid on foliage treated in the field with Sevin XLR and Imidan 70WSB than emerged from the water treated control. Sevin XLR remained toxic for 7 d in the field while the effects of Imidan 70WSB were no longer significant after one week. The number of *A. vaquitarum* emerging from host eggs laid on treated foliage was not significantly different among Micromite 80WGS, Acramite 50WS and the control, but significantly fewer adults emerged from foliage treated with either Micromite 80WGS + Oil or Oil alone. Though not toxic to *A. vaquitarum*, treatment with Oil appears to result in premature opening of *D. abbreviatus* egg masses which deters females from ovipositing and desiccates parasitoid eggs and larvae. There were no significant differences between oviposition or new generation adults for females exposed to Micromite 80WGS and those exposed to a water control.

Delta-9 Desaturase from the Diaprepes Root Weevil. *C.S. Katsar, W.B. Hunter, and S.L. Lapointe. USDA, ARS, U.S. Horticultural Research Lab, Fort Pierce, FL 34945.*

Diaprepes root weevils (DRW) cost citriculture millions of dollars in lost production. DRW has spread to Texas, and is a threat to California agriculture. New strategies to monitor and limit DRW damage to crops are needed. More effective trapping and management strategies depend upon identification of an attractant or pheromone for this species. Fatty acid desaturases are enzymes that catalyze the insertion of a double bond at the Δ9 position of fatty acids. Unsaturated fatty acids are important constituents of all cell membranes and are required for normal insect growth and pheromone synthesis. A full length cDNA was cloned from the DRW and the protein characterized *in silico* to determine if pheromone precursors were present.

Results of a Three-Year-Field Study of Surround® Particle Film for Control of the Diaprepes Root Weevil. *S.L. Lapointe and C.L. McKenzie. USDA, ARS, U.S. Horticultural Research Lab, Fort Pierce, FL 34945.*

Regularly applied sprays of a particle film (Surround® WP) greatly enhanced the growth of citrus trees on poorly drained Winder soil at Fort Pierce, FL. After 3 years of applications every 3 or 4 every weeks, Surround-treated trees had at least 5 times the mass, 6 times the canopy volume, and approximately 4 times the cross-sectional area of the tree stems at the graft union compared to untreated trees. The larger Surround-treated trees attracted a higher number of Diaprepes root weevil adults per tree, but an equivalent number of egg masses per tree compared with the control trees. The results suggest that oviposition per adult was reduced on Surround-treated trees. The number of larvae per tree recovered from the roots of excavated trees was greater from Surround-treated trees compared with control trees. While Surround particle film greatly enhanced growth of citrus trees grown in a poorly drained soil where trees were stressed by edaphic factors, the deterrence to oviposition by *D. abbreviatus* was insufficient to significantly reduce the number of root weevil larvae per tree feeding on the roots. However, the more vigorous trees resulting from Surround applications may be more resistant or tolerant to root weevil feeding.

IPM Using Entomopathogenic Nematodes: The Roles of Longevity and Adaptation. *L.W. Duncan,[1] K. Nguyen,[2] and F.E. El-Borai.[1] [1]UF-IFAS, Citrus REC, Lake Alfred, FL 33850. [2]Department of Entomology and Nematology, Gainesville, FL 32611.*

We tested the hypothesis that the rate of insect biocontrol is positively related to the persistence of entomopathogenic nematodes (EPN) used to augment EPN communities. Individual citrus trees were treated (25 IJ cm^{-2}) with *Steinernema riobrave* (Sr; short persistence), *S. diaprepesi* (Sd; long persistence), or no EPN in September and October 2002, May and September 2003, and April and August 2004. Mortality of sentinel *Diaprepes abbreviatus* larvae was monitored 11 times between July 2003 and October 2004. Fourteen days post-treatment, weevil mortality in plots treated with Sd and Sr was as high as 83% and 72%, respectively, compared to 35% in untreated plots. The average mortality of sentinel larvae in plots treated with Sd (39%) was 38% higher than in untreated plots (26%) and 25% higher than in plots treated with Sr (29%) (P=0.05). Average recycling of EPN in insect cadavers was greater (P=0.01) in plots treated with Sd (30%) than in plots treated with Sr (16%) and in untreated plots (20%). Reproduction by free-living bactivorous nematodes in insect cadavers was greater (P=0.01) in plots treated with Sr (9.3%) than in plots treated with Sd (4.0%) and untreated plots (3.7%). In this experiment, augmentation of the longer-lived Sd provided more effective biocontrol than augmentation with Sr; however, declining EPN prevalence in Sd-augmented plots in autumn 2003 suggested that laboratory adaptation by Sd may occur at the expense of traits needed for effective predation and persistence in soil. Also, Sr was the most prominent EPN in the untreated control plots (not treated with Sr for more than 2 years prior to this experiment) suggesting that the exotic Sr is adapting to Florida habitats. We compared populations of Sd and Sr isolated from this orchard (wild) to populations of both species reared in commercial laboratories for at least 2 years. Longevity and efficacy did not differ among wild and commercial isolates of either species in pasteurized soil. The wild isolate of Sd persisted significantly longer and killed more weevils in unpasteurized soil than did the commercial isolate.

Toward Circumventing Non-Target Effects of Augmenting Entomopathogenic Nematode Communities. *L.W. Duncan,[1] J.H. Graham,[1] F. El-Borai,[1] and D.L. Porazinska.[2] [1]UF-IFAS, Citrus REC, Lake Alfred, FL 33850. [2]Ft. Lauderdale REC, Fort Lauderdale, FL 33314.*

To be effective, chemical pesticides must be used in ways that mitigate non-target effects that create secondary pests, pest resurgence, accelerated microbial degradation, and pesticide resistance. Non-target effects of augmenting soil with entomopathogenic nematodes are virtually unknown; however, there is also no evidence that they are less prevalent or important than those involving chemical pesticides. We are testing the hypothesis that natural enemies of entomopathogenic nematodes (EPN) increase in response to EPN augmentation and subsequently reduce biological control of insect pests to lower than normal levels. Two weeks after augmenting the EPN beneath citrus trees to control larvae of *Diaprepes abbreviatus,* there were significant increases in mortality of sentinel weevil larvae, prevalence of nematophagous fungi, and prevalence of free-living bactivorous nematodes in cadavers of sentinel weevils. Six weeks following EPN augmentation, significantly fewer sentinel weevil larvae died in augmented compared to non-augmented plots. Application of composted manure as a mulch layer beneath trees decreased the prevalence of nematophagous fungi, increased the prevalence of endemic EPN, and increased the mortality of sentinel weevil larvae. In the laboratory, addition of *S. riobrave* to soil from the orchard increased the mortality of both *S. riobrave* and *S. diaprepesi* that were added to the soil 7 d later. At the end of 2 weeks, greater numbers of nematophagous fungi and fewer EPN remained in soil that was augmented at the beginning of weeks one and two, than in soil that was augmented only in week two. These effects did not occur in soil that was air-dried to disrupt fungal activity prior to the experiment. *Heterorhabditis zealandica* was significantly less affected by pre-augmentation than were the steinernematid species. Apparently, effects of the post-application biology of EPN on biological control can be modulated in important ways by the choice of augmentation timing, EPN species, and cultural practices.

Ant Predation on Neonate Larvae of the Root Weevil, *Diaprepes abbreviatus*: A Manipulative Field Experiment. *R.J. Stuart and C W. McCoy. UF-IFAS CREC, Lake Alfred, FL 33850.*

The root weevil, *Diaprepes abbreviatus* (L.), continues to be a major pest of Florida citrus. When *D. abbreviatus* neonate larvae hatch from egg masses in the citrus canopy and drop to the soil surface before burrowing down to the roots for feeding, they are extremely vulnerable to ant predation. In Florida citrus groves, the red imported fire ant, *Solenopsis invicta* Buren, is one of the most common ant species, and previous research indicates that it is also one of the most important predators of *Diaprepes* neonates. We conducted a manipulative field experiment to examine the relationship between ant population levels and predation pressure on *Diaprepes* neonates. Granular ant baits were used to suppress ant populations in a young citrus grove. Ant populations were monitored by placing hamburger baits under the citrus canopy; and predation pressure on *Diaprepes* neonates was assessed by placing neonates in plastic dishes under the canopy. In this experiment, *S. invicta* was the major predator on *Diaprepes* neonates, and there was a significant correlation between ant population levels and predation rates on neonates. This research reinforces the view that red imported fire ants are major natural enemies in Florida citrus groves and important predators of *Diaprepes* neonates.